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(54) Title: DNA AND AMINO ACID SEQUENCE SPECIFIC FOR NATURAL KILLER CELLS (57) Abstract The invention relates to DNA sequences and to the corresponding proteins, which are expressed in NK cells and some T cells. The protein is a transmembrane molecule with a type II membrane protein structure. The extracellular part of the receptor is characterised by a C-type animal lectin domain. Antibodies against the extracellular part of the transmembrane protein can activate the NK-cells and T-cells.		

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**DNA AND AMINO ACID SEQUENCE SPECIFIC
FOR NATURAL KILLER CELLS**

This invention was made with partial government support under R 01 AI19007 awarded by the National Institutes of Health. The government has certain rights in the invention.

This invention relates to DNA and cDNA sequences which are normally transcribed in natural killer (NK) cells or in some T cells, vectors comprising the DNA or cDNA sequences and the proteins encoded by the DNA or cDNA sequences. The DNA sequences can be detected by employing a combination of differential hybridisation and cDNA subtraction methodology.

Natural killer (NK) cells are present in the peripheral blood in a state capable of lysing NK sensitive targets in a reaction that is not restricted by proteins of the major histocompatibility complex. These cells typically account for about 5% of peripheral blood lymphocytes and they usually possess the large granular lymphocyte (LGL) morphology. In human systems NK activity is usually defined as the ability to lyse cells of the promyeloid leukaemia, K562. In many cases stimulation of NK cells with interleukin 2 (IL-2) will confer on them lymphokine activated killer (LAK) activity, the ability to lyse a

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broader range of tumour target cells. Many ongoing studies are attempting to use cells with LAK activity in anti-tumour therapy.

From mRNA isolated from human NK cells it has been possible to generate a cDNA library from which has been isolated four related novel genes which constitute a new mammalian gene family, hereinafter designated NKG2. In sequence homology searches using standard selectivity criteria and the Intelligenetics software program to search the SwissProt and PIR databases, significant homologies were detected only with members of the type II transmembrane proteins with C-type animal lectin domains, and not with any other sequences. Although the overall sequence homology is low, they share homology throughout a stretch of approximately 120 amino acids with the most striking feature being the presence of six invariant cysteine residues at fixed positions. In the known transmembrane proteins, this region corresponds to a carbohydrate binding domain which enables the proteins to bind carbohydrates in a Ca^{++} and pH-dependent manner. (Drickamer 1988 J Biol Chem 263, 9557). It is therefore believed that the novel gene family of the present invention also codes for transmembrane proteins having a carbohydrate bonding domain.

The invention provides the genes coding for the extracellular part of four specific NKG2 transmembrane proteins, as well as for the complete transmembrane proteins. The invention also provides the corresponding proteins, hereinafter designated NKG2-A, -B, -C and -D.

A murine molecule belonging to the gene family of type II membrane proteins is described by Yokoyama (Yokoyama et al 1989 J. Immunol. 143). The murine Ly-49 alloantigen is recognised by the A1 monoclonal antibody, and is a type II membrane protein, the expression of which is confined almost exclusively to a subset of mouse NK cells. The gene is found to map to the same position on mouse chromosome six as the gene for NK1.1, a commonly used murine NK cell marker also expressed almost exclusively on NK cells. Ly-49 is expressed on only about 20% of NK1.1 positive cells and is distinct from NK1.1.

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A further molecule first identified by a special monoclonal antibody is described by Chambers (Chambers et al 1989 J Exp Med 169, 1373). This molecule corresponds to the antigen NKR-P1 and is expressed almost exclusively on rat NK cells. The reaction of this antibody with its target antigen delivers a transmembrane signal as evidenced by its ability to mediate reverse antibody-dependent cellular cytotoxicity of an appropriate Fc receptor positive target cell. The corresponding cDNA encodes a type II membrane protein of 223 amino acids. (Giorda et al 1990 Science 249, 1298)

Although the Ly-49 and NKR-P1 antigen genes have similarities with NKG2, they display very limited amino acid sequence homology, the homology with the NKG2-A protein sequence being 33% for Ly49 and 23% for NKR-P1. It is accordingly most unlikely that NKG2 is simply the human equivalent of these murine genes (in which case an amino acid homology of approx. 70% or more would be expected), but rather represents a novel gene family.

The novel genes are preferentially expressed in NK cells and T cells of mammals, preferably of primates and most preferably of humans. By using a standard method such as Northern blot technique the mRNA corresponding to the genes of the invention can be detected in some T cells, but cannot be detected in B cells, on EBV-transformed B cell lines (EBV = Epstein-Barr virus) or other cells.

The present invention provides new DNA or cDNA sequences and novel proteins having the corresponding amino acid sequences, all of these being in isolated pure form. By "isolated pure form" is meant in a form substantially (>90%, preferably >95%) free of other DNA or protein of mammalian origin.

In particular, the invention provides an isolated DNA or cDNA molecule encoding the extracellular part of a transmembrane protein herein designated (a) NKG2-A_f, (b) NKG2-B_f, (c) NKG2-C_f and (d) NKG2-D_f (f = fragment) translated in natural killer cells or T cells,

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wherein the molecule contains a DNA or cDNA sequence selected from the group comprising:

- a) that part of the DNA sequence shown in Seq. Id. No.: 1, comprising the nucleotides numbered 458 to 863 inclusive (= the entire sequence shown in Seq. Id. No.: 3); or
- b) that part of the DNA sequence shown in Seq. Id. No. 1, comprising the nucleotides numbered 503 to 863 inclusive (= the entire sequence shown in Seq. Id. No. 4); or
- c) that part of the DNA sequence shown in Seq. Id. No.: 5 comprising the nucleotides numbered 296 to 700 inclusive (= the entire sequence shown in Seq. Id. No.: 7); or
- d) that part of the DNA sequence shown in Seq. Id. No.: 8, comprising the nucleotides numbered 585 to 986 inclusive (= the entire sequence shown in Seq. Id. No.: 10).

Additionally the invention comprises DNA or cDNA sequences that code for the same amino acid sequence as that coded for by any of a)-d) above; and/or which hybridise under stringent conditions to any of a)-d) above; and/or have a homology of 80 to 100% with any of the DNA sequences a)-d) above. A homology of 90 to 100% is preferred, more preferred is a homology of 95 to 100%, particularly preferred is 98 to 100%.

The activity of the extracellular part of the proteins comprises inter alia the recognition of a ligand on a cell surface, and there is evidence that said ligand is a carbohydrate structure which is for example expressed on cancer cells and virus infected cells (Drickamer 1988, J Biol Chem 263: 9557).

Additionally the invention provides an isolated DNA or cDNA molecule encoding a complete transmembrane protein herein designated

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aa) NKG2-A, bb) NKG2-B, cc) NKG2-C and dd) NKG2-D which are translated in natural killer cells or T cells, wherein the molecule contains a DNA or cDNA sequence selected from the group comprising:

- aa) that part of the DNA sequence shown in Seq. Id. No.: 1, comprising the nucleotides numbered 165 to 863 inclusive (= the entire sequence shown in Seq. Id. No.: 11), or
- bb) that part of the DNA sequence shown in Seq. Id. No. 1, comprising the nucleotides numbered 165 to 863 inclusive; the nucleotides numbered 449 to 502 being deleted (= the entire sequence shown in Seq. Id. No.: 12); or
- cc) that part of the DNA sequence shown in Seq. Id. No.: 5 comprising the nucleotides numbered 8 to 700 inclusive (= the entire sequence shown in Seq. Id. No.: 13); or
- dd) that part of the DNA sequence shown in Seq. Id. No.: 8, comprising the nucleotides numbered 339 to 986 inclusive (= the entire sequence shown in Seq. Id. No.: 14).

The deletion referred to in (bb) interrupts two codons, TC...T (Ser) and GC...A (Ala), but there is no effect upon the translation as the codon formed after the deletion, TCA, also codes for serine.

Additionally the invention comprises isolated DNA or cDNA sequences that code for the same amino acid sequence as that coded for by any of aa)-dd) above; and/or which hybridise under stringent conditions to any of aa)-dd) above; and/or have a homology of 80 to 100% with any of the DNA sequences aa)-dd) above. A homology of 90 to 100% is preferred, more preferred is a homology of 95 to 100%, particularly preferred is 98 to 100%.

The complete transmembrane protein is considered to be a receptor molecule the function of which comprises inter alia:

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- 1) the recognition of a ligand on a cell surface, whereby said ligand may be a carbohydrate structure which is for example expressed on cancer cells and virus infected cells (but other structures are not excluded);
- 2) the transmission of a signal from the extracellular space into the cytoplasm;
- 3) the activation of NK or T cells by such signal resulting in the induction of cytotoxicity and/or proliferation.

Preferred DNA or cDNA sequences are those whose degree of homology with the reference DNA sequence are as given above, calculated on the basis of the total number of nucleotide changes in the coding region. Less preferred are those whose degree of homology is within the above ranges when calculated on the basis of only those nucleotide changes which give rise to a change in amino acid sequence.

The invention also includes DNA or cDNA sequences comprising the coding sequence from aa), bb), cc) or dd) and additionally a noncoding sequence. The complete sequences of the invention are shown in the Seq. Id. 1, 5, 8, and 15. The given DNA sequences code for human proteins. Homologous DNA sequences coding for corresponding proteins found in other mammalian species are also preferred sequences.

A DNA sequence of the invention may be a cDNA recombinant DNA sequence which may be present in the genetic material of a host cell or non human organism. The DNA of the invention can be transferred into prokaryotic or eucaryotic cells. The sequence of one of the Seq. Id. No.: 1, 5, 8, or 15 is combined with a suitable promoter such as tac-promoter and introduced into a suitable vector such as pGEX-2T or pKK233-2, which is then used to transform a prokaryotic cell such as *E. coli* (for example strain: Mol42). The sequence of one of the Seq. Id. No.: 1, 5, 8, or 15 can also be directly introduced into a eukaryotic cell. Preferred cells are CHO cells or primate cells such as COS, because these latter cells guarantee the correct type of glycosylation. For optimum transcription in mammalian cells a

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suitable promoter has to be used with a compatible vector, for example CMV promoter in a pCDM8 vector.

The invention also provides the extracellular parts of the four transmembrane proteins encoded by the novel gene family, these extracellular parts being designated a') NKG2-A_f, b') NKG2-B_f, c') NKG2-C_f and d') NKG2-D_f (f=fragment) having amino acid sequences selected from the group comprising:

- a') that part of the amino acid sequence in Seq. Id. Nos.: 1 and 2 comprising the amino acids numbered 99 to 223 (= the entire amino acid sequence of Seq. Id. No.: 17); or
- b') that part of the amino acid sequence in Seq. Id. No.: 1 comprising the amino acids numbered 114 to 233 (= the entire amino acid sequence of Seq. Id. No.: 18); or
- c') that part of the amino acid sequence in Seq. Id. No.: 5 comprising the amino acids numbered 97 to 231 (= the entire amino acid sequence of Seq. Id. No.: 19); or
- d') that part of the amino acid sequence in Seq. Id. No.: 9 comprising the amino acids numbered 83 to 216 (= the entire amino acid sequence of Seq. Id. No.: 20).

Additionally the invention provides the isolated complete transmembrane proteins encoded by the novel gene family, designated aa') NKG2-A, bb') NKG2-B, cc') NKG2-C and dd') NKG2-D, having amino acid sequences selected from the group comprising:

- aa') the amino acid sequence in Seq. Id. No.: 2 (or Seq. Id. No.: 21); or
- bb') the amino acid sequence in Seq. Id. No.: 2 with the deletion of the amino acids numbered 96 to 113 (or Seq. Id. No.: 22);

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or

cc') the amino acid sequence in Seq. Id. No.: 6 (or Seq. Id. No.: 23); or

dd') the amino acid sequence in Seq. Id. No.: 8 (or Seq. Id. No.: 24).

Knowing the nucleotide sequences of the invention the person skilled in the art can synthesise the DNA according to the general procedure described in PCT Publication WO 90/07861. (Synthesis of oligonucleotides, amplification by the PCR reaction and splicing to from the complete DNA sequence). Once the DNA sequence has been obtained it can be further amplified and introduced into a host cell for transcription and translation of the protein. The protein can be expressed, isolated and purified by standard methods.

The proteins of the invention show a considerable degree of similarity. The proteins each comprise an extracellular, a transmembrane and an intracellular segment. The transmembrane segments include the following amino acids: Seq. Id. No.: 2 = from 71 to 98 inclusive or from 71 to 95 inclusive; Seq. Id. No.: 6 = from 71 to 96 inclusive and Seq. Id. No.: 8 = from 52 to 82 inclusive.

The protein NKG2-B (Seq. Id. No.: 1 including deletion or Seq. Id. No.: 15) is identical to NKG2-A (Seq. Id. No.: 1) except for the absence of an 18 amino acid segment immediately outside the transmembrane region. NKG2-C (Seq. Id. 5) shows the strongest homology (94%) with the extracellular segment of NKG2-A and lesser homology (56%) throughout the intracellular and transmembrane segments, giving an overall homology of 76%. NKG2-D (Seq. Id. 8) displays distant but significant homology (21%) with NKG2-A, NKG2-B and NKG2-C. Fig. 1 shows a diagram of the regions of homology in the DNA sequences of the transcripts. The shaded areas refer to the percent homology between the adjacent transcripts. The stippled region within the NKG2-A,

NKG2-B and NKG2-C transcripts is 95% homologous with the 5' end of NKG2-D.

The proteins of the invention have similar functions to those of the known type II integral membrane proteins. The proteins deliver transmembrane signals. The extracellular C-terminus and the intracellular N-terminus are connected by a hydrophobic region capable of serving as a transmembrane domain. The extracellular segment can be activated by specific molecules, for example monoclonal antibodies specifically recognising the extracellular segment. By forming a complex between the inducing molecule and the protein of the invention the three dimensional structure of the protein of the invention is altered. The intracellular segment changes its configuration, the lytic activity is increased. When the protein of the invention is recognised by the inducing molecule the killer function is activated.

The present invention includes not only proteins having exactly the same amino acid sequence as those described above, but also proteins which are sequence variants of the above proteins. A "sequence variant" is defined herein as a protein which has a homology of at least 70%, preferably at least 80%, more preferably at least 90%, particularly at least 95%, with one of the proteins a')-d'); or a homology of at least 80%, preferably at least 90%, more preferably at least 95%, with one of the proteins aa')-dd'); and essentially the same biological properties as one of the proteins a')-d') or aa')-dd').

By "essentially the same biological properties" is meant that the sequence variant protein corresponding to the extracellular domain of the transmembrane protein or to the complete transmembrane protein must be capable of binding to a ligand molecule on an NK target cell, and that the complete transmembrane protein must be capable of transmitting a signal across the membrane.

Amino acid sequence variants of the inventive proteins include,

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for example, deletions from, or insertions or substitutions f, residues within the unmodified amino acid sequences. Any combination of deletion, insertion and substitution may also be made to arrive at the final construct, provided that the final construct possesses the desired activity. Obviously, any mutations made in the DNA encoding the variants must not place the sequence out of reading frame.

Amino acid sequence deletions generally range from about 1 to 30 residues, more preferably 1 to 10 residues, and typically are contiguous. Amino acid sequence insertions include amino- and/or carboxy-terminal fusions of from one residue to polypeptides of essentially unrestricted length, as well as intrasequence insertions of single or multiple amino acid residues. Intrasequence insertions may range generally from about 1 to 10 residues, more preferably 1 to 5.

The proteins of the invention very often consist of sequences with at least one altered amino acid residue compared with the amino acid sequence of the Sequence Identifier (referring to the amino acids) of the invention. Preferably only one has been removed and a different residue inserted in its place. Such substitutions generally are made in accordance with the following Table I when it is desired to modulate finely the characteristics of the proteins of the invention.

Substantial changes in function are made by selecting substitutions that are less conservative than those in Table I, i.e., selecting residues that differ more significantly in their effect on maintaining (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the receptor binding site, or (c) the bulk of the side chain.

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Table I

Original Residues

Exemplary Substitutions

Ala	Gly, Ser
Arg	Lys
Asn	Gln, His
Asp	Glu
Cys	Ser
Gln	Asn
Glu	Asp
Gly	Ala, Pro
His	Asn, Gln
Ile	Leu, Val
Leu	Ile, Val
Lys	Arg, Gln, Glu
Met	Leu, Tyr, Ile
Phe	Met, Leu, Tyr
Ser	Thr
Thr	Ser
Trp	Tyr
Tyr	Trp, Phe
Val	Ile, Leu

Most deletions and insertions, and substitutions in particular, are not expected to produce radical changes in the characteristics of the proteins of the inventions. However, it is difficult to predict the exact effect of the substitution, deletion or insertion in advance of doing so, for example, when modifying the the receptor binding domain.

As the NKG2 proteins act as transmembrane proteins whose function it is to transmit a signal to activate the NK cell, it is believed that the NK immune response will be strengthened if the number of NKG2 protein molecules on the surface of the NK cell is increased.

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In order to increase the number of expressed NKG2 molecules, the natural killer cells of patients suffering from cancer or viral disease may be isolated and transformed by the DNA of the invention. NK cells are obtainable from the blood, lymph node or spleen cells of a patient, and these cells may be transformed in vitro and re-injected into the patient. The isolated DNA sequence is introduced into patient-derived NK cells using known techniques of transfection for example by retroviral vectors. The protein encoded by such introduced DNA does not differ from person to person, unlike many other cell proteins (Major Histocompatibility Complex Antigen). Therefore an uncontrolled attack of the transformed cells against self antigens is excluded and the transformed cells themselves will not be attacked by the immune system.

The proteins of the invention may be obtained as the products of recombinant expression, using essentially standard methods, as outlined above and described in more detail below. The protein may be purified for example by the use of a polyclonal or monoclonal antibody which has been raised against a specific peptide sequence characteristic of the protein which it is desired to isolate.

The isolated proteins of the invention, preferably the extracellular domains, are useful as a diagnostic tool for detecting target ligands for example carbohydrate groups which are present on cancer cells and some virus infected cells.

The isolated complete transmembrane protein is a suitable tool for the study of the mechanism of NK cell regulation. Additionally the natural three dimensional structure of the protein, which is part of a synthetic or natural membrane, makes it possible to test different chemical compounds which have an influence on the change of configuration to block or to enhance the triggering of the function of the transmembrane protein.

Additionally the proteins of the invention, preferably the extra-

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cellular parts of the proteins, may be linked to a cytotoxic molecule, for example ricin, to form a chimeric protein molecule capable of lysis of cancer and virus infected cells.

The purified proteins of the invention are also useful as antigens to produce monoclonal or polyclonal antibodies against the proteins, using standard methods which are well known to the person skilled in the art. Alternatively, instead of immunising animals such as mice with the purified antigen, cells bearing the protein molecules on the membrane may be used as immunogen. Using these methods antibodies are established against epitopes of the protein, the extracellular epitopes being preferred. The protein may be offered as an antigen on cells of a different species (e.g. human protein on murine syngeneic cells) to enrich the specific B cell population producing antibodies against the antigen. The murine cells are transformed by introducing the coding part of the human DNA of the invention preferably combined with a suitable promoter into the genetic material of the mouse cell. Then the transformed cells are injected into a syngeneic mouse as a highly specific immunogen. After producing hybridomas by fusion according to standard methods the monoclonal antibodies produced by the hybridomas are screened for example by ELISA with original (human) NK cells or with pure protein.

Further a monoclonal antibody or domain specifically recognising the proteins of the invention may be combined with a second monoclonal antibody or binding domain recognising a specific structure on cancer cells or virus-infected cells. A binding domain is part of at least one protein which recognises parts (for example epitopes) of the protein of the invention. A typical binding domain is the variable region of the heavy or light chain of an antibody. Such combined molecules or binding domains are useful for the treatment of specific forms of cancer. The production of bifunctional antibodies is described for example in J. Van Dijk et al (1989) Int J of Cancer 44: 738-743.

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In particular, the α -NKG2-mAbs or α NKG2- α -cancer-mAb-complexes cause an increase in the activity of human natural killer cells against human tumor cells, as shown in the $^{51}\text{[Cr]}$ release test described by Brunner et al. (1981) J Exp Med 154: 362 - 373. Tumor cells as target cells which are labelled by $^{51}\text{[Cr]}$ are incubated in the presence of NK cells for 4 to 6 hours. The effectiveness of the NK cells is measured by the release of $^{51}\text{[Cr]}$ label into the medium. The $^{51}\text{[Cr]}$ release is increased in the presence of the antibody or antibody complex according to the invention. Activity against gibbon tumour cells are tested in the same in vitro system with the exception that gibbon NK cells are used.

The free extracellular proteins of the invention, i.e. proteins aa')-dd'), are useful agents for blocking the ligand molecules on the NK target cells and are therefore useful as immunosuppressants in situations such as organ transplantation and autoimmune disease.

For these indications the appropriate dosage varies depending upon, for example, the host, the mode of administration and the nature and severity of the condition being treated. In general, satisfactory results in animals are obtained at daily dosages from about 20 μg to about 15 mg, preferably from 150 μg to 2 mg per kg body weight and per day. The dosages are administered in a single dose or in divided dosages up to four times per day.

The compounds of the invention are administered by any conventional route, in particular by injection in solution or suspension in a pharmaceutically acceptable diluent or carrier. Such compositions are manufactured in conventional manner.

Description of the drawing:

Fig. 1 shows a diagram of the regions of homology in the DNA sequences of the proteins of the invention, namely NKG2-A, NKG2-B, NKG2-C and NKG2-D. The shaded areas refer to the percent homology between the adjacent transcribed mRNAs. The stippled region within the NKG2-A,

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NKG2-B and NKG2-C transcripts is 95% homologous with the 5' end of NKG2-D. Above the four lines representing the mRNAs a translated protein is shown comprising the extracellular, the transmembrane and the intracellular part of the molecule.

EXAMPLES

Cell Culture

The cloned human NK cell, B22 (CD3-, CD16-, CD56+), and the NK cell populations EDF (CD3-, CD16+, CD56-) and 221707 (CD3-, CD16+, CD56+) derived from NK cell leukocytosis patients are cultured in RPMI 1640 medium containing 15 % pooled human serum, 20 % T cell growth factor (TCGF) (Biotest, FRG), 2 mM L-glutamine, supplemented with streptomycin and penicillin. The cells are stimulated weekly with irradiated (10,000 Rad) LCL feeder cells (LCL = Lymphoblastoid cell line).

Cells are harvested and RNA isolated on day 6 or 7 of the growth cycle. Frozen PBL (= peripheral blood lymphocytes) from the patients are depleted for CD3+ cells (which constitute less than 15 % of the original samples) by treatment with OKT3 and rabbit complement. The EDF culture is treated with LCL feeder cells at two week intervals and cells are harvested after 3 weeks of culture. 221707 is not treated with feeder cells and is harvested after 2 weeks of culture. After complement depletion of CD3+ cells and growth for 2 to 3 weeks in TCGF-containing medium, virtually 100 % of the cells in the population have the NK phenotype. Allogeneic cytotoxic T cell clones (Tc) are cultured in the same medium as the NK cell lines and are stimulated weekly with LCL feeder cells. Tc1 and Tc2 are CD4+ and Tc3 is CD8+. The lymphoblastoid cell line, FJO, and the leukaemic T cell line, Jurkat, are cultured in RPMI 1640 containing 10 % foetal calf serum, glutamine and antibiotics.

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The CD4-positive all geneic T cell clones, KD15, .3-78, KD33, and a CD4-positive cytomegalovirus-specific helper T cell clone, WRC-16, are cultured in the same medium as the NK clone. The chronic myelogenous leukaemia line, K562, the histocytic lymphoma line, U937, the Jurkat cell line, and FJO cell line are cultured in RPMI 1640 containing 10 % foetal calf serum, glutamine and antibiotics. The T cell lymphoma line, Hut78, is cultured in the same medium as above, with the addition of 10 % TCGF. The promyelocytic leukaemia line, HL60, is cultured in the same medium as above except that it contains 20 % foetal calf serum. One half of the HL60 is stimulated with 1.25 % DMSO at days 1 and 3 and harvested at day 7. DMSO (dimethyl sulfoxide) stimulation induces approximately 50 % of the cells to differentiate into more mature myeloid cell forms. The monocyte line, THP-1, is cultured in RPMI 1640 containing 10 % foetal calf serum and $2 \times 10^{-5}M$ β -mercaptoethanol.

NK cell activation

B22, normally maintained in 20 % TCGF, is resuspended in medium containing 5 % TCGF, in which cells remain viable but do not proliferate. After two days, the cells are returned to medium containing 20 % TCGF. Total cytoplasmic RNA is extracted at various time points and subjected to Northern blot analysis.

Isolation and sequencing of NKG2 cDNA clones

12 independent cDNA clones are isolated from the B22 cDNA library using differential hybridisation as described in the following.

cDNA Library Preparation

Messenger RNA used for preparation of cDNA libraries, Northern blots, or cDNA probe is extracted and eluted from oligo dT cellulose using the method of JE Badley et al. (1988): BioTechniques 6, 114. cDNA libraries of B22 and FJO are prepared using the method of

Palazzolo & Meyerovitz (1987): Gene 52, 197. Briefly, 2 µg poly(A) RNA is converted to double-stranded cDNA using the Bethesda Research Laboratories cDNA Synthesis System, except that first-strand synthesis is primed with an oligonucleotide having an XbaI cloning site at its 5' end and a homopolymeric T tail at its 3' end. The double-stranded cDNA is methylated with EcoRI methylase, ligated to EcoRI linkers, and digested with EcoRI and XbaI. The cDNA is then size fractionated on a Biogel A50M column and all fractions with a minimum size of 400 bp are combined, ligated to EcoRI-XbaI arms of the Lambda (λ) GEM-2 vector (Promega Biotech), and packaged using Gigapack Gold (Stratagene). A library of 10⁶ primary plaques is amplified. The final libraries consist of cDNA inserts with an XbaI site adjacent to an SP6 RNA polymerase promoter at the 3' end of the message and an EcoRI site next to a T7 RNA polymerase promoter at the 5' end.

λ-DNA from libraries or clones is prepared from plate lysates using the polyethylene glycol/NaCl precipitation method described by T Maniatis et al. (1982) Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. DNA preparations from the B22 and FJ0 libraries are subsequently used to prepare a subtracted cDNA library following the method of MJ Palazzolo et al. (1989): Neurone 3, 527. After digestion of library DNA with XbaI, T7 RNA polymerase is used to synthesise poly(A) RNA (hereafter referred to as synthetic RNA) which is immediately loaded onto an oligo (dT) cellulose column, and bound fractions containing approximately 80 % of the newly synthesised RNA are recovered.

³²[P]-labelled first-strand cDNA is prepared from the B22 synthetic RNA using the same priming oligomer described above, and the RNA is subsequently base hydrolyzed. The annealing procedure and subsequent steps are carried out twice starting either with 2 or 6 µg of B22 first-strand cDNA. The cDNA is twice annealed to a 10-20 fold excess of FJ0 synthetic RNA at 42° for 72 hours in the presence of 40 % formamide, 0.5 M sodium phosphate (pH 6.8), 10 mM EDTA and 0.2 % SDS (sodium dodecylsulfate). After each annealing, the sample is applied

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to a BioRad HPHT HPLC column equilibrated with 10 mM sodium phosphate (pH 6.8) at 60°. Non-annealed, single-stranded cDNA is eluted with 154 mM phosphate buffer, and fractions containing cDNA are combined. After two cycles of subtraction approximately 0.2 µg single-stranded NK cDNA is recovered. An oligonucleotide homologous to a short vector encoded stretch at the 3' end of the subtracted first-strand cDNA is used to prime second-strand synthesis. The double-stranded cDNA is then cleaved with EcoRI and XbaI, size fractionated on a Biogel A50M column and ligated into EcoRI-XbaI λGEM-2 arms. A total of 1100 plaques are obtained from the two trials after in vitro packaging and plating. Each plaque is picked with a glass capillary tube and the plug transferred to a well of a 96-well plate containing 100 µl of SM buffer.

Plaque Hybridisation Studies

Differential hybridisation is performed on plaque lifts prepared on nitro-cellulose membranes. During the first differential screening of the total library, lifts are prepared from 150 mm plates containing 500-800 plaques. All subsequent screenings are performed on ordered plaques prepared using a sterile stainless steel transfer device that duplicates the ordered array of a 96-well plate. First strand ^{32}P -labelled cDNA probe is prepared from mRNA using the same conditions of cDNA synthesis described above except that dCTP is present at 25 µM and (α - ^{32}P)dCTP is present at 3.5 µCi/µl. RNA is base hydrolyzed and cDNA is separated from unincorporated label on a small Sephadex G50 column. Previously described gene probes are labelled using either the Nick Translation Kit or the Multiprime Labelling System from Amersham. Probes prepared from individual clones for cross-hybridization studies are of several types. In some cases, DNA inserts are excised from individual clones, electrophoresed on a 1.3 % low melting point agarose gel and the insert band recovered from the gel and labelled using the multiprime labelling system of Amersham. Alternatively, one ng of λ-DNA from a clone is amplified by PCR using the GeneAmp system from Perkin Elmer Cetus. The amplified product is

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again purified on a 1.3 % low melting point agarose gel and labelled using the Multiprime system. Plaque hybridisations with all DNA probes are carried out as in Maniatis et al. (Gyllenstein, U.B & Erlich, H.A. (1988) Proc Natl Acad Sci USA 85, 7652). Asymmetric PCR is used to amplify inserts and the 5'-end sequence (up to 450 nucleotides in length) is determined for seven of the clones. A full length cDNA sequence is determined by subcloning PstI fragments encompassing the entire NKG2 insert into M13mp19.

NKG2 cDNA probes

The NKG2 cDNA fragments are labelled using the multiprime system from Amersham. Labelled NKG2 cDNA fragments are used as probes in Northern blot hybridisation and plaque hybridisation.

Northern Blot Studies

RNA samples are isolated as described above. At appropriate time points after exposure to 20 % TCGF, cells are lysed with a low concentration of NP40, nuclei are pelleted and the aqueous phase extracted with phenol/chloroform. Northern analysis is performed as described by P Thomas (1980) Proc. Natl. Acad. Sci. USA 77, 5201. Briefly, RNA samples are denatured and applied to formaldehyde-agarose denaturing gels. The RNA is transferred from the gel to GeneScreen Plus (DuPont) by capillary blotting. Blots are prehybridised (1M NaCl, 50 % formamide, 10 % dextran sulphate, 5x Denhardt's and 1 % SDS) for 12-16 hours at 42 °C. Labelled denatured DNA probe is added to the blot at 1×10^6 cpm/ml (cpm = counts per minute) hybridisation buffer, and hybridisation continued for 24 hours at 42 °C. The blots are then washed and exposed for autoradiography.

Labelled NKG2 cDNA probes are mixed with denatured salmon sperm DNA (35 µg/ml hybridisation solution) and then added to the blot at $1-5 \times 10^6$ cpm/ml hybridisation buffer. Following overnight hybridis-

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ation at 42°C, blots are washed two times in 2 x SSC for 5 min. at room temperature, then twice in 2 x SSC and 1 % SDS for 30 min. at 50°C.

Southern blot hybridisation

Genomic DNA is digested with the indicated restriction enzyme and 10 µg/lane of digested DNA is electrophoresed on a 0.7 % agarose gel. The DNA is transferred to nitro-cellulose membrane by capillary blotting using 20 x SSC. Hybridisation and washes are performed as in Nicklas, J.A., et al. (1985) Human Immunol. 13, 95.

DNA Sequencing

Polymerase chain reaction amplifications used to prepare single-stranded DNA (Gyllenstein & Erlich) for sequencing contain in 100 µl final volume: 1 ng λ-DNA, 50 mM Tris/HCl pH 8.8, 10 mM MgCl₂, 10 mM (NH₄)₂SO₄, 1.5 mM of each dNTP (= deoxynucleotide-triphosphate), 100 moles of SP6 polymerase promoter primer, 1 pmole T7 polymerase promoter primer (both from Promega), and 2.5 units Taq polymerase (Perkin Elmer Cetus). Samples are cycled 35 times through 1 min. denaturation at 94 °C, 2 min. annealing at 49 °C, and 3 min. extension at 72 °C. Following the amplification, samples are purified using Millipore Ultrafree-MC low binding filter units (10,000 mol weight cut-off). Approximately one-half of the amplified sample is then used for DNA sequencing which is carried out with standard protocols for Sequenase (USB) or Taq Track (Promega) if secondary structural problems are apparent. DNA sequences up to 450 nucleotides in length are obtained by this method. DNA sequence comparison with GenBank and EMBL databases is performed with the Intelligenetics FastDB program.

Formation of Antibodies to the Protein

Peptides are synthesised corresponding to a) the 15 C-terminal amino acids of NKG2-C and b) the 15 amino acids of position 157-171 of NKG2-C. This latter sequence forms part of the lectin domain, and is

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predicted to constitute surface exposed helices. Both peptides are synthesised with an additional cysteine on their N-terminus, conjugated to KLH and used for immunization of several rabbits according to standard procedures. Antibodies raised against the peptides are purified from the rabbit sera via a peptide column.

Production of Recombinant Protein

Expression of Complete Protein in Insect Cells

Recombinant NKG2-C protein is produced in the baculovirus system according to published procedures (Invitrogen Manual). The NKG2-C cDNA is cloned into the pVL1393 baculovirus expression vector and Sf9 insect cells are cotransfected with the plasmid and wild type viral DNA. Recombinant virus, which contains the NKG2-C DNA integrated into the viral genome by homologous recombination of the plasmid with the wild type viral DNA, is selected by four rounds of plaque purification. Recombinant virus is scored on filter replicas obtained from infected cells. Following purification of the recombinant virus from wild type viruses, infections of Sf9 cells are performed at different virus to cell ratios. The production of recombinant NKG2-C protein is determined after lysis of the infected cells in buffers containing 1% triton X-100. Identical amounts of protein are separated on polyacrylamide gels, the proteins electrophoretically transferred to Immobilon-P membranes and NKG2-C detected by its reactivity in Western blots with the polyclonal anti-peptide antibodies. No product is detected in this way in controls infected only with wild type virus. The estimated molecular weight in PAGE is consistent with the predicted value of 26 kD.

Expression of Extracellular Domain in E coli

The cDNA for NKG2-C is cut with the BstNI restriction endonuclease at the start of the region encoding the extracellular domain, and the fragment cloned into the EcoRI site of the pFlag1 plasmid. This

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construct directs the secretion of a flag-NKG2-C fusion protein to the periplasmic space with the help of the ompA signal peptide. The flag is an eight amino acid tag-sequence added for the purpose of easier purification only. Extracts from E coli obtained after osmotic shock of the cells is used for protein transfer blots with the antibodies described above. A protein of approx. 17 kD reactive with the antibody is found in the extract from the transfected E coli but not in control extracts obtained from parallel cultures transfected with the expression vector without a NKG2-C insert.

Purification of Recombinant Protein

Crude protein isolated by lysis of Sf9 cells from a large-scale fermentation of transfected cells is purified on an immunoaffinity column the stationary phase of which is bonded to one of the two anti-NKG2-C peptide antibodies described above. The column is then eluted at pH 3-4 to remove the desired protein from the column, and the protein is further purified using standard techniques for example ion-exchange and reverse-phase chromatography. The purity of the product can be estimated by SDS-PAGE.

Crude protein from fermentation of E coli is purified in similar manner using on the immunoaffinity column a commercially available MAb to the flag peptide. The eluted protein is specifically cleaved from the flag peptide by enterokinase, and purified by conventional methods.

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1387 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 165..863

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```

GCAGGCATTG TTTGTTCTTG TCTTGGATTT ATGCCTTTAA ATTTCACCTT TTATTACACA      60
GCTATAGCAG GCCTTTTAT GAGACTAACC TGGCCTCTCC ACTAAAGGAT GTGTGACTTT      120
CTGGGGACAG AAGAGTACAG TCCCTGACAT CACACACTGC AGAG ATG GAT AAC CAA      176
                                   Met Asp Asn Gln
                                   1
GGA GTA ATC TAC TCA GAC CTG AAT CTG CCC CCA AAC CCA AAG AGG CAG      224
Gly Val Ile Tyr Ser Asp Leu Asn Leu Pro Pro Asn Pro Lys Arg Gln
  5                               10                               15                               20
CAA CGA AAA CCT AAA GGC AAT AAA AGC TCC ATT TTA GCA ACT GAA CAG      272
Gln Arg Lys Pro Lys Gly Asn Lys Ser Ser Ile Leu Ala Thr Glu Gln
                25                               30                               35
GAA ATA ACC TAT GCG GAA TTA AAC CTT CAA AAA GCT TCT CAG GAT TTT      320
Glu Ile Thr Tyr Ala Glu Leu Asn Leu Gln Lys Ala Ser Gln Asp Phe
                40                               45                               50
CAA GGG AAT GAC AAA ACC TAT CAC TGC AAA GAT TTA CCA TCA GCT CCA      368
Gln Gly Asn Asp Lys Thr Tyr His Cys Lys Asp Leu Pro Ser Ala Pro
                55                               60                               65
GAG AAG CTC ATT GTT GGG ATC CTG GGA ATT ATC TGT CTT ATC TTA ATG      416
Glu Lys Leu Ile Val Gly Ile Leu Gly Ile Ile Cys Leu Ile Leu Met
  70                               75                               80

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GCC TCT GTG GTA ACG ATA GTT GTT ATT CCC TCT ACA TTA ATA CAG AGG 464
 Ala Ser Val Val Thr Ile Val Val Ile Pro Ser Thr Leu Ile Gln Arg
 85 90 95 100

CAC AAC AAT TCT TCC CTG AAT ACA AGA ACT CAG AAA GCA CGT CAT TGT 512
 His Asn Asn Ser Ser Leu Asn Thr Arg Thr Gln Lys Ala Arg His Cys
 105 110 115

GGC CAT TGT CCT GAG GAG TGG ATT ACA TAT TCC AAC AGT TGT TAC TAC 560
 Gly His Cys Pro Glu Glu Trp Ile Thr Tyr Ser Asn Ser Cys Tyr Tyr
 120 125 130

ATT GGT AAG GAA AGA AGA ACT TGG GAA GAG AGT TTG CTG GCC TGT ACT 608
 Ile Gly Lys Glu Arg Arg Thr Trp Glu Glu Ser Leu Leu Ala Cys Thr
 135 140 145

TCG AAG AAC TCC AGT CTG CTT TCT ATA GAT AAT GAA GAA GAA ATG AAA 656
 Ser Lys Asn Ser Ser Leu Leu Ser Ile Asp Asn Glu Glu Glu Met Lys
 150 155 160

TTT CTG TCC ATC ATT TCA CCA TCC TCA TGG ATT GGT GTG TTT CGT AAC 704
 Phe Leu Ser Ile Ile Ser Pro Ser Ser Trp Ile Gly Val Phe Arg Asn
 165 170 175 180

AGC AGT CAT CAT CCA TGG GTG ACA ATG AAT GGT TTG GCT TTC AAA CAT 752
 Ser Ser His His Pro Trp Val Thr Met Asn Gly Leu Ala Phe Lys His
 185 190 195

GAG ATA AAA GAC TCA GAT AAT GCT GAA CTT AAC TGT GCA GTG CTA CAA 800
 Glu Ile Lys Asp Ser Asp Asn Ala Glu Leu Asn Cys Ala Val Leu Gln
 200 205 210

GTA AAT CGA CTT AAA TCA GCC CAG TGT GGA TCT TCA ATA ATA TAT CAT 848
 Val Asn Arg Leu Lys Ser Ala Gln Cys Gly Ser Ser Ile Ile Tyr His
 215 220 225

TGT AAG CAT AAG CTT TAGAGGTAAA GCGTTTGCAT TTGCAGTGCA TCAGATAAAT 903
 Cys Lys His Lys Leu
 230

TGTATATTTT TAAAAATAGA AATATATTAT GATTGCATAA ATCTTAAAAAT GAATTATGTT 963

ATTTGCTCTA ATAAGAAAAT TCTAAATCAA TTATTGAAAC AGGATACACA CAATTACTAA 1023

AGTACAGACA TCCTAGCATT TGTGTCGGGC TCATTTTGCT CAACATGGTA TTTGTGGTTT 1083

TCAGCCTTTC TAAAAGTTGC ATGTTATGTG AGTCAGCTTA TAGGAAGTAC CAAGAACAGT 1143

CAAACCCATG GAGACAGAAA GTAGAATAGT GGTGCGCAAT GTCTGAGGGA GGTGAAATA 1203

GGAGATGACC TCTAACTGAT AGAACGTTAC TTTGTGTCGT GATGAAAAC TTTCTAAATTT 1263
 CAGTAGTGGT GATGGITGTA ACTCTGCGAA TATACTAAAC ATCATTGATT TTTAATCATT 1323
 TTAAGTGCAT GAAATGTATG CTTTGTACAC GACACTTCAA TAAAGCTATC CAGAAAAAAA 1383
 AAAA 1387

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 233 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Asp Asn Gln Gly Val Ile Tyr Ser Asp Leu Asn Leu Pro Pro Asn
 1 5 10 15
 Pro Lys Arg Gln Gln Arg Lys Pro Lys Gly Asn Lys Ser Ser Ile Leu
 20 25 30
 Ala Thr Glu Gln Glu Ile Thr Tyr Ala Glu Leu Asn Leu Gln Lys Ala
 35 40 45
 Ser Gln Asp Phe Gln Gly Asn Asp Lys Thr Tyr His Cys Lys Asp Leu
 50 55 60
 Pro Ser Ala Pro Glu Lys Leu Ile Val Gly Ile Leu Gly Ile Ile Cys
 65 70 75 80
 Leu Ile Leu Met Ala Ser Val Val Thr Ile Val Val Ile Pro Ser Thr
 85 90 95
 Leu Ile Gln Arg His Asn Asn Ser Ser Leu Asn Thr Arg Thr Gln Lys
 100 105 110
 Ala Arg His Cys Gly His Cys Pro Glu Glu Trp Ile Thr Tyr Ser Asn
 115 120 125
 Ser Cys Tyr Tyr Ile Gly Lys Glu Arg Arg Thr Trp Glu Glu Ser Leu
 130 135 140

Leu Ala Cys Thr Ser Lys Asn Ser Ser Leu Ieu Ser Ile Asp Asn Glu
 145 150 155 160
 Glu Glu Met Lys Phe Leu Ser Ile Ile Ser Pro Ser Ser Trp Ile Gly
 165 170 175
 Val Phe Arg Asn Ser Ser His His Pro Trp Val Thr Met Asn Gly Leu
 180 185 190
 Ala Phe Lys His Glu Ile Lys Asp Ser Asp Asn Ala Glu Leu Asn Cys
 195 200 205
 Ala Val Leu Gln Val Asn Arg Leu Lys Ser Ala Gln Cys Gly Ser Ser
 210 215 220
 Ile Ile Tyr His Cys Lys His Lys Leu
 225 230

(2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 405 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

CAGAGGCACA ACAATTCCTC CCTGAATACA AGAACTCAGA AAGCACGTCA TTGTGGCCAT 60
 TGTCTGAGG AGTGGATTAC ATATTCCAAC AGTTGTTACT ACATTGGTAA GGAAAGAAGA 120
 ACTTGGGAAG AGAGTTTGCT GGCCTGTACT TCGAAGAACT CCAGTCTGCT TTCTATAGAT 180
 AATGAAGAAG AAATGAAATT TCTGTCCATC ATTTCAACCAT CCTCATGGAT TGGTGTGTTT 240
 CGTAACAGCA GTCATCATCC ATGGGTGACA ATGAATGGTT TGGCTTTCAA ACATGAGATA 300

AAAGACTCAG ATAATGCTGA ACTTAACTGT GCAGTGCTAC AAGTAAATCG ACTTAAATCA 360
GCCCCAGTGTG GATCTTCAAT AATATATCAT TGTAAGCATA AGCTT 405

(2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 306 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

TACATTGGTA AGGAAAGAAG AACTTGGGAA GAGAGTTTGC TGGCCTGTAC TTCCAAGAAC 60
TCCAGTCTGC TTTCTATAGA TAATGAAGAA GAAATGAAAT TTCTGTCCAT CATTTCACCA 120
TCCTCATGGA TTGGTGTGTT TCGTAACAGC AGTCATCATC CATGGGTGAC AATGAATGGT 180
TTGGCTTTCA AACATGAGAT AAAAGACTCA GATAATGCTG AACTTAACTG TGCAGTGCTA 240
CAAGTAAATC GACTTAAATC AGCCCAAGTGT GGATCTTCAA TAATATATCA TTGTAAGCAT 300
AAGCTT 306

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1222 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 8..700

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

TGCAGAG ATG AGT AAA CAA AGA GGA ACC TTC TCA GAA GTG AGT CTG GCC	49
Met Ser Lys Gln Arg Gly Thr Phe Ser Glu Val Ser Leu Ala	
1 5 10	
CAG GAC CCA AAG CGG CAG CAA AGG AAA CCT AAA GGC AAT AAA AGC TCC	97
Gln Asp Pro Lys Arg Gln Gln Arg Lys Pro Lys Gly Asn Lys Ser Ser	
15 20 25 30	
ATT TCA GGA ACC GAA CAG GAA ATA TTC CAA GTA GAA TTA AAT CTT CAA	145
Ile Ser Gly Thr Glu Gln Glu Ile Phe Gln Val Glu Leu Asn Leu Gln	
35 40 45	
AAT CCT TCC CTG AAT CAT CAA GGG ATT GAT AAA ATA TAT GAC TGC CAA	193
Asn Pro Ser Leu Asn His Gln Gly Ile Asp Lys Ile Tyr Asp Cys Gln	
50 55 60	
GGT TTA CTG CCA CCT CCA GAG AAG CTC ACT GCC GAG GTC CTA GGA ATC	241
Gly Leu Leu Pro Pro Pro Glu Lys Leu Thr Ala Glu Val Leu Gly Ile	
65 70 75	
ATT TGC ATT GTC CTG ATG GCC ACT GTG TTA AAA ACA ATA GTT CTT ATT	289
Ile Cys Ile Val Leu Met Ala Thr Val Leu Lys Thr Ile Val Leu Ile	
80 85 90	

CCT TTC CTG GAG CAG AAC AAT TCT TCC CCG AAT ACA AGA ACG CAG AAA	337
Pro Phe Leu Glu Gln Asn Asn Ser Ser Pro Asn Thr Arg Thr Gln Lys	
95 100 105 110	
GCA CGT CAT TGT GGC CAT TGT CCT GAG GAG TGG ATT ACA TAT TCC AAC	385
Ala Arg His Cys Gly His Cys Pro Glu Glu Trp Ile Thr Tyr Ser Asn	
115 120 125	
AGT TGT TAT TAC ATT GGT AAG GAA AGA AGA ACT TGG GAA GAG AGT TTG	433
Ser Cys Tyr Tyr Ile Gly Lys Glu Arg Arg Thr Trp Glu Glu Ser Leu	
130 135 140	
CTG GCC TGT ACT TCG AAG AAC TCC AGT CTG CTT TCT ATA GAT AAT GAA	481
Leu Ala Cys Thr Ser Lys Asn Ser Ser Leu Leu Ser Ile Asp Asn Glu	
145 150 155	
GAA GAA ATA AAA TTT CTG GCC AGC ATT TTA CCT TCC TCA TGG ATT GGT	529
Glu Glu Ile Lys Phe Leu Ala Ser Ile Leu Pro Ser Ser Trp Ile Gly	
160 165 170	
GTG TTT CGT AAC AGC AGT CAT CAT CCA TGG GTG ACA ATA AAT GGT TTG	577
Val Phe Arg Asn Ser Ser His His Pro Trp Val Thr Ile Asn Gly Leu	
175 180 185 190	
GCT TTC AAA CAT AAG ATA AAA GAC TCA GAT AAT GCT GAA CTT AAC TGT	625
Ala Phe Lys His Lys Ile Lys Asp Ser Asp Asn Ala Glu Leu Asn Cys	
195 200 205	
GCA GTG CTA CAA GTA AAT CGA CTT AAA TCA GCC CAG TGT GGA TCT TCA	673
Ala Val Leu Gln Val Asn Arg Leu Lys Ser Ala Gln Cys Gly Ser Ser	
210 215 220	
ATG ATA TAT CAT TGT AAG CAT AAG CTT TAGAAGTAAA GCATTTGCGT	720
Met Ile Tyr His Cys Lys His Lys Leu	
225 230	
TTACAGTGCA TCAGATACAT TTTATATTTT TTAATAAGTA AATATTATGA TTGCATAAAT	780
CTGAAAATGA ATTATGTTAT TTGCTCTAAT AAAAAAATTC TAAATCAATT ATTGAAATAG	840
GATGCACACA ATTACTAAAG TACAGACATC CTAGCATTG TGTCGGGCTC ATTTTGCTCA	900
ACATGGTATT TGTGGTTTTT AGCCTTTCTA AAAGTTGCAT GTTATGTCAG TCAGCTTATA	960
GGAAGTACCA AGAACAGTCA AACCCATGGA GACAGAAAGT AGAATAGTGG TTGCCAATGT	1020
CTCAGGGAGG TTGAAATAGG AGATGACCAC TAATTGATAG AACGTTTCTT TGTGTCGTGA	1080

TGAAGACTTT CTAAATTTCA GTAATGGTGA TGGTTGTAAC TTGCGAATA TACTAAACAT 1140
 CATTGATTTT TAATCATTTT AAGTGCATGA AATGTATGCT TTGTACATGA CACTTCAATA 1200
 AAGCTATCCA GAAAAA AAAA AA 1222

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 231 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met	Ser	Lys	Gln	Arg	Gly	Thr	Phe	Ser	Glu	Val	Ser	Leu	Ala	Gln	Asp	1	5	10	15
Pro	Lys	Arg	Gln	Gln	Arg	Lys	Pro	Lys	Gly	Asn	Lys	Ser	Ser	Ile	Ser	20	25	30	
Gly	Thr	Glu	Gln	Glu	Ile	Phe	Gln	Val	Glu	Leu	Asn	Leu	Gln	Asn	Pro	35	40	45	
Ser	Leu	Asn	His	Gln	Gly	Ile	Asp	Lys	Ile	Tyr	Asp	Cys	Gln	Gly	Leu	50	55	60	
Leu	Pro	Pro	Pro	Glu	Lys	Leu	Thr	Ala	Glu	Val	Leu	Gly	Ile	Ile	Cys	65	70	75	80
Ile	Val	Leu	Met	Ala	Thr	Val	Leu	Lys	Thr	Ile	Val	Leu	Ile	Pro	Phe	85	90	95	
Leu	Glu	Gln	Asn	Asn	Ser	Ser	Pro	Asn	Thr	Arg	Thr	Gln	Lys	Ala	Arg	100	105	110	
His	Cys	Gly	His	Cys	Pro	Glu	Glu	Trp	Ile	Thr	Tyr	Ser	Asn	Ser	Cys	115	120	125	
Tyr	Tyr	Ile	Gly	Lys	Glu	Arg	Arg	Thr	Trp	Glu	Glu	Ser	Leu	Leu	Ala	130	135	140	
Cys	Thr	Ser	Lys	Asn	Ser	Ser	Leu	Leu	Ser	Ile	Asp	Asn	Glu	Glu	Glu	145	150	155	160

Ile Lys Phe Leu Ala Ser Ile Leu Pro Ser Ser Trp Ile Gly Val Phe
 165 170 175
 Arg Asn Ser Ser His His Pro Trp Val Thr Ile Asn Gly Leu Ala Phe
 180 185 190
 Lys His Lys Ile Lys Asp Ser Asp Asn Ala Glu Leu Asn Cys Ala Val
 195 200 205
 Leu Gln Val Asn Arg Leu Lys Ser Ala Gln Cys Gly Ser Ser Met Ile
 210 215 220
 Tyr His Cys Lys His Lys Leu
 225 230

(2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 405 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CTGGAGCAGA ACAATTCCTC CCCGAATACA AGAACGCAGA AAGCACGTCA TTGTGGCCAT 60
 TGTCTGAGG AGTGGATTAC ATATTCCAAC AGTTGTTATT ACATTGGTAA GGAAAGAAGA 120
 ACTTGGGAAG AGAGTTTGCT GGCCTGTACT TCGAAGAACT CCAGTCTGCT TTCTATAGAT 180
 AATGAAGAAG AAATAAAATT TCTGGCCAGC ATTTTACCTT CCTCATGGAT TGGTGTGTTT 240
 CGTAACAGCA GTCATCATCC ATGGGTGACA ATAAATGGTT TGGCTTTCAA ACATAAGATA 300
 AAAGACTCAG ATAATGCTGA ACTTAACTGT GCAGTGCTAC AAGTAAATCG ACTTAAATCA 360
 GCCCAGTGTG GATCTTCAAT GATATATCAT TGTAAGCATA AGCTT 405

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1755 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 339..986

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

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GAGGAGTGGG TTACATATTC CAACAGTTGT TATTACATTG GTAAGGAAAG AAGAACTTGG 60
GAAGAAAGAG TTGCTGGCC TGIGCTTCGA AGAACTCTGA TCTGCTTCT ATAGATAATG 120
AGGAAGAAAT GGTATGTGTG GGGACTTCCC AGTTGGCTGT AAGTTGCCAT TTGAACTAAA 180
CGAAATAGAT CAGGAAGTGA GGACATATCT AAATTTTCTA GTTTTATAGA AGGCTTTTAT 240
CCACAAGAAT CAAGATCTTC CCTCTCTGAG CAGGAATCCT TTGTGCATTG AAGACTTTAG 300
ATTCCTCTCT GCGGTAGACG TGCACTTATA AGTATTTG ATG GGG TGG ATT CGT 353
                               Met Gly Trp Ile Arg
                               1       5

GGT CGG AGG TCT CGA CAC AGC TGG GAG ATG AGT GAA TTT CAT AAT TAT 401
Gly Arg Arg Ser Arg His Ser Trp Glu Met Ser Glu Phe His Asn Tyr
          10          15          20

AAC TTG GAT CTG AAG AAG AGT GAT TTT TCA ACA CGA TGG CAA AAG CAA 449
Asn Leu Asp Leu Lys Lys Ser Asp Phe Ser Thr Arg Trp Gln Lys Gln
          25          30          35

AGA TGT CCA GTA GTC AAA AGC AAA TGT AGA GAA AAT GCA TCT CCA TTT 497
Arg Cys Pro Val Val Lys Ser Lys Cys Arg Glu Asn Ala Ser Pro Phe
          40          45          50

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TTT TTC TGC TGC TTC ATC GCT GTA GCC ATG GGA ATC CGT TTC ATT ATT 545
 Phe Phe Cys Cys Phe Ile Ala Val Ala Met Gly Ile Arg Phe Ile Ile
 55 60 65

ATG GTA GCA ATA TGG AGT GCT GTA TTC CTA AAC TCA TTA TTC AAC CAA 593
 Met Val Ala Ile Trp Ser Ala Val Phe Leu Asn Ser Leu Phe Asn Gln
 70 75 80 85

GAA GTT CAA ATT CCC TTG ACC GAA AGT TAC TGT GGC CCA TGT CCT AAA 641
 Glu Val Gln Ile Pro Leu Thr Glu Ser Tyr Cys Gly Pro Cys Pro Lys
 90 95 100

AAC TGG ATA TGT TAC AAA AAT AAC TGC TAC CAA TTT TTT GAT GAG AGT 689
 Asn Trp Ile Cys Tyr Lys Asn Asn Cys Tyr Gln Phe Phe Asp Glu Ser
 105 110 115

AAA AAC TGG TAT GAG AGC CAG GCT TCT TGT ATG TCT CAA AAT GCC AGC 737
 Lys Asn Trp Tyr Glu Ser Gln Ala Ser Cys Met Ser Gln Asn Ala Ser
 120 125 130

CTT CTG AAA GTA TAC AGC AAA GAG GAC CAG GAT TTA CTT AAA CTG GTG 785
 Leu Leu Lys Val Tyr Ser Lys Glu Asp Gln Asp Leu Leu Lys Leu Val
 135 140 145

AAG TCA TAT CAT TGG ATG GGA CTA GTA CAC ATT CCA ACA AAT GGA TCT 833
 Lys Ser Tyr His Trp Met Gly Leu Val His Ile Pro Thr Asn Gly Ser
 150 155 160 165

TGG CAG TGG GAA GAT GGC TCC ATT CTC TCA CCC AAC CTA CTA ACA ATA 881
 Trp Gln Trp Glu Asp Gly Ser Ile Leu Ser Pro Asn Leu Leu Thr Ile
 170 175 180

ATT GAA ATG CAG AAG GGA GAC TGT GCA CTC TAT GCC TCG AGC TTT AAA 929
 Ile Glu Met Gln Lys Gly Asp Cys Ala Leu Tyr Ala Ser Ser Phe Lys
 185 190 195

GGC TAT ATA GAA AAC TGT TCA ACT CCA AAT ACA TAC ATC TGC ATG CAA 977
 Gly Tyr Ile Glu Asn Cys Ser Thr Pro Asn Thr Tyr Ile Cys Met Gln
 200 205 210

AGG ACT GTG TAAAGATGAT CAACCATCTC AATAAAAGCC AGGAACAGAG 1026
 Arg Thr Val
 215

AAGAGATTAC ACCAGCGGTA ACACTGCCAA CCGAGACTAA AGGAAACAAA CAAAAACAGG 1086

ACAAATGAC CAAAGACTGT CAGATTTCTT AGACTCCACA GGACCAAACC ATAGAACAAT 1146

TTCCTGCAA ACATGCATGA TTCTCCAAGA CAAAAGAAGA GAGATCCTAA AGGCAATTCA 1206

GATAATCCCCA AGGCTGCCTC TCCCACCACA AGCCCAGAGT GGATGGGCTG GGGGAGGGGT 1266
 GCTGTTTAA TTTCTAAAGG TAGGACCAAC ACCCAGGGGA TCACTGAAGG AAGAGAAGGC 1326
 CAGCAGATCA GTGAGAGTGC AACCCACCC TCCACAGGAA ATTGCCTCAT GGGCAGGGCC 1386
 ACAGCAGAGA GACACAGCAT GGGCAGTGCC TTCCCTGCCT GTGGGGGTCA TGCTGCCACT 1446
 TTTAATGGGT CCTCCACCCA ACGGGGTCAG GGAGGTGGTG CTGCCCCAGT GGGCCATGAT 1506
 TATCTTAAAG GCATTATTCT CCAGCCTTAA GATCTTAGGA CGTTTCCTTT GCTATGATTT 1566
 GTACTTGCTT GAGTCCCATG ACTGTTTCTC TTCCTCTCTT TCTTCCTTTT GGAATAGTAA 1626
 TATCCATCCT ATGTTTGTCC CACTATTGTA TTTTGAAGC ACATAACTTG TTTGGTTTCA 1686
 CAGGTCACA GTTAAGAAGG AATTTTGCCT CTGAATAAAT AGAATCTTGA GTCTCATGCA 1746
 AAAAAAAAAA 1755

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 216 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Met Gly Trp Ile Arg Gly Arg Arg Ser Arg His Ser Trp Glu Met Ser
 1 5 10 15
 Glu Phe His Asn Tyr Asn Leu Asp Leu Lys Lys Ser Asp Phe Ser Thr
 20 25 30
 Arg Trp Gln Lys Gln Arg Cys Pro Val Val Lys Ser Lys Cys Arg Glu
 35 40 45
 Asn Ala Ser Pro Phe Phe Phe Cys Cys Phe Ile Ala Val Ala Met Gly
 50 55 60
 Ile Arg Phe Ile Ile Met Val Ala Ile Trp Ser Ala Val Phe Leu Asn
 65 70 75 80

Ser	Leu	Phe	Asn	Gln	Glu	Val	Gln	Ile	Pro	Leu	Thr	Glu	Ser	Tyr	Cys
			85						90					95	
Gly	Pro	Cys	Pro	Lys	Asn	Trp	Ile	Cys	Tyr	Lys	Asn	Asn	Cys	Tyr	Gln
			100					105					110		
Phe	Phe	Asp	Glu	Ser	Lys	Asn	Trp	Tyr	Glu	Ser	Gln	Ala	Ser	Cys	Met
		115					120					125			
Ser	Gln	Asn	Ala	Ser	Leu	Leu	Lys	Val	Tyr	Ser	Lys	Glu	Asp	Gln	Asp
	130					135					140				
Leu	Leu	Lys	Leu	Val	Lys	Ser	Tyr	His	Trp	Met	Gly	Leu	Val	His	Ile
145					150					155					160
Pro	Thr	Asn	Gly	Ser	Trp	Gln	Trp	Glu	Asp	Gly	Ser	Ile	Leu	Ser	Pro
				165					170					175	
Asn	Leu	Leu	Thr	Ile	Ile	Glu	Met	Gln	Lys	Gly	Asp	Cys	Ala	Leu	Tyr
			180					185					190		
Ala	Ser	Ser	Phe	Lys	Gly	Tyr	Ile	Glu	Asn	Cys	Ser	Thr	Pro	Asn	Thr
		195					200					205			
Tyr	Ile	Cys	Met	Gln	Arg	Thr	Val								
	210					215									

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 402 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

TTCAACCAAG AAGTTCAAAT TCCCTTGACC GAAAGTTACT GTGGCCCATG TCCTAAAAAC	60
TGGATATGTT AAAAAATAA CTGCTACCAA TTTTTCATG AGAGTAAAA CTGGTATGAG	120
AGCCAGGCTT CTGTATGTC TCAAATGCC AGCCTTCTGA AAGTATACAG CAAAGAGGAC	180
CAGGAITTC TAAACTGGT GAAGTCATAT CATTGGATGG GACTAGTACA CATTCCAACA	240
AATGGATCTT GGCAGTGGGA AGATGGCTCC ATTCTCTCAC CCAACCTACT AACATAATT	300
GAAATGCAGA AGGGAGACTG TGCACTCTAT GCCTCGAGCT TTAAAGGCTA TATAGAAAAC	360
TGTTCAACTC CAAATACATA CATCTGCATG CAAAGGACTG TG	402

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 699 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

ATGGATAACC AAGGAGTAAT CTACTCAGAC CTGAATCTGC CCCCCAACCC AAAGAGGCAG	60
CAACGAAAAC CTAAAGGCAA TAAAAGCTCC ATTTTAGCAA CTGAACAGGA AATAACCTAT	120
GCGGAATTAA ACCTTCAAAA AGCTTCTCAG GATTTTCAAG GGAATGACAA AACCTATCAC	180
TGCAAAGATT TACCATCAGC TCCAGAGAAG CTCATTGTTG GGATCCTGGG AATTATCTGT	240
CTTATCTTAA TGGCCTCTGT GGTAACGATA GTTGTTATTC CCTCTACATT AATACAGAGG	300
CACAACAATT CTCCCTGAA TACAAGAACT CAGAAAGCAC GTCATTGTGG CCATTGTCCT	360
GAGGAGTGGA TTACATATTC CAACAGTTGT TACTACATTG GTAAGGAAAG AAGAACTTGG	420
GAAGAGAGTT TGCTGGCCTG TACTTCGAAG AACTCCAGTC TGCTTTCTAT AGATAATGAA	480
GAAGAAATGA AATTTCTGTC CATCATTTCA CCATCCTCAT GGATTGGTGT GTTTCGTAAC	540
AGCAGTCATC ATCCATGGGT GACAATGAAT GGTTTGGCTT TCAAACATGA GATAAAAGAC	600
TCAGATAATG CTGAACTTAA CTGTGCAGTG CTACAAGTAA ATCGACTTAA ATCAGCCCAG	660
TGTGGATCTT CAATAATATA TCATTGTAAG CATAAGCTT	699

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 645 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

ATGGATAACC AAGGAGTAAT CTACTCAGAC CTGAATCTGC CCCCAAACCC AAAGAGGCAG	60
CAACGAAAAC CTAAAGGCAA TAAAGCTCC ATTTTAGCAA CTGAACAGGA AATAACCTAT	120
GCGGAATTAA ACCTTCAAAA AGCTTCTCAG GATTTTCAAG GGAATGACAA AACCTATCAC	180
TGCAAAGATT TACCATCAGC TCCAGAGAAG CTCATTGTTG GGATCCTGGG AATTATCTGT	240
CTTATCTTAA TGGCCTCTGT GGTAACGATA GTTGTTATTC CCTCACGTCA TTGTGGCCAT	300
TGTCCTGAGG AGTGGATTAC ATATTCCAAC AGTTGTTACT ACATTGGTAA GGAAAGAAGA	360
ACTTGGGAAG AGAGTTTGCT GGCCTGTACT TCGAAGAACT CCAGTCTGCT TTCTATAGAT	420
AATGAAGAAG AAATGAAATT TCTGTCCATC ATTTCAACAT CCTCATGGAT TGGTGTGTTT	480
CGTAACAGCA GTCATCATCC ATGGGTGACA ATGAATGGTT TGGCTTTCAA ACATGAGATA	540
AAAGACTCAG ATAATGCTGA ACTTAACTGT GCAGTGCTAC AAGTAAATCG ACTTAAATCA	600
GCCCAGTGTG GATCTTCAAT AATATATCAT TGTAAGCATA AGCTT	645

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 693 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

ATGAGTAAAC AAAGAGGAAC CTTCTCAGAA GTGAGTCTGG CCCAGGACCC AAAGCGGCAG	60
CAAAGGAAAC CTAAAGGCAA TAAAGCTCC ATTTCAGGAA CCGAACAGGA AATATTCCAA	120
GTAGAATTAA ATCTTCAAAA TCCTTCCTG AATCATCAAG GGATTGATAA AATATATGAC	180
TGCCAAGGTT TACTGCCACC TCCAGAGAAG CTCACTGCCG AGGTCCTAGG AATCATTTCG	240
ATTGTCCTGA TGGCCACTGT GTTAAAAACA ATAGTTCTTA TTCCTTTCCT GGAGCAGAAC	300
AATTCTTCCC CGAATACAAG AACGCAGAAA GCACGTCATT GTGGCCATTG TCCTGAGGAG	360
TGGATTACAT ATTCCAACAG TTGTTATTAC ATTGGTAAGG AAAGAAGAAC TTGGGAAGAG	420
AGTTTGCTGG CCTGTACTTC GAAGAACTCC AGTCTGCTTT CTATAGATAA TGAAGAAGAA	480
ATAAAATTC TGGCCAGCAT TTTACCTTCC TCATGGATTG GTGTGTTTCG TAACAGCAGT	540
CATCATCCAT GGGTGACAAT AAATGGTTTG GCTTTCAAAC ATAAGATAAA AGACTCAGAT	600
AATGCTGAAC TTAAGTGTGC AGTGCTACAA GTAAATCGAC TTAAATCAGC CCAGTGTGGA	660
TCTTCAATGA TATATCATTG TAAGCATAAG CTT	693

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 648 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

ATGGGGGTGGA	TTCGTGGTCG	GAGGTCTCGA	CACAGCTGGG	AGATGAGTGA	ATTCATAAT	60
TATAACTTGG	ATCTGAAGAA	GAGTGATTTT	TCAACACGAT	GGCAAAAGCA	AAGATGTCCA	120
GTAGTCAAAA	GCAAATGTAG	AGAAAATGCA	TCTCCATTTT	TTTTCTGCTG	CTTCATCGCT	180
GTAGCCATGG	GAATCCGTTT	CATTATTATG	GTAGCAATAT	GGAGTGCTGT	ATTCCTAAAC	240
TCATTATTCA	ACCAAGAAGT	TCAAATTCCC	TTGACCGAAA	GTTACTGTGG	CCCATGTCCT	300
AAAAACTGGA	TATGTTACAA	AAATAACTGC	TACCAATTTT	TTGATGAGAG	TAAAACTGG	360
TATGAGAGCC	AGGCTTCTTG	TATGTCTCAA	AATGCCAGCC	TTCTGAAAGT	ATACAGCAAA	420
GAGGACCAGG	ATTACTTAA	ACTGGTGAAG	TCATATCATT	GGATGGGACT	AGTACACATT	480
CCAACAAATG	GATCTTGGCA	GTGGGAAGAT	GGCTCCATTC	TCTCACCCAA	CCTACTAACA	540
ATAATTGAAA	TGCAGAAGGG	AGACTGTGCA	CTCTATGCCT	CGAGCTTTAA	AGGCTATATA	600
GAAAACTGTT	CAACTCCAAA	TACATACATC	TGCATGCAAA	GGACTGTG		648

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1333 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 165...809

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

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GCAGGCATTG TTTGTTCTTG TCTTGATT ATGCCTTTAA ATTCACCTT TTATTACACA 60
GCTATAGCAG GCCTTTTAT GAGACTAACC TGGCCTCTCC ACTAAAGGAT GTGTGACTTT 120
CTGGGGACAG AAGAGTACAG TCCCTGACAT CACACACTGC AGAG ATG GAT AAC CAA 176
                               Met Asp Asn Gln
                               1
GGA GTA ATC TAC TCA GAC CTG AAT CTG CCC CCA AAC CCA AAG AGG CAG 224
Gly Val Ile Tyr Ser Asp Leu Asn Leu Pro Pro Asn Pro Lys Arg Gln
 5              10              15              20
CAA CGA AAA CCT AAA GGC AAT AAA AGC TCC ATT TTA GCA ACT GAA CAG 272
Gln Arg Lys Pro Lys Gly Asn Lys Ser Ser Ile Leu Ala Thr Glu Gln
          25              30              35
GAA ATA ACC TAT GCG GAA TTA AAC CTT CAA AAA GCT TCT CAG GAT TTT 320
Glu Ile Thr Tyr Ala Glu Leu Asn Leu Gln Lys Ala Ser Gln Asp Phe
          40              45              50
CAA GGG AAT GAC AAA ACC TAT CAC TGC AAA GAT TTA CCA TCA GCT CCA 368
Gln Gly Asn Asp Lys Thr Tyr His Cys Lys Asp Leu Pro Ser Ala Pro
          55              60              65
GAG AAG CTC ATT GTT GGG ATC CTG GGA ATT ATC TGT CTT ATC TTA ATG 416
Glu Lys Leu Ile Val Gly Ile Leu Gly Ile Ile Cys Leu Ile Leu Met
          70              75              80

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GCC TCT GTG GTA ACG ATA GTT GIT ATT CCC TCA CGT CAT TGT GGC CAT	464
Ala Ser Val Val Thr Ile Val Val Ile Pro Ser Arg His Cys Gly His	
85 90 95 100	
TGT CCT GAG GAG TGG ATT ACA TAT TCC AAC AGT TGT TAC TAC ATT GGT	512
Cys Pro Glu Glu Trp Ile Thr Tyr Ser Asn Ser Cys Tyr Tyr Ile Gly	
105 110 115	
AAG GAA AGA AGA ACT TGG GAA GAG AGT TTG CTG GCC TGT ACT TCG AAG	560
Lys Glu Arg Arg Thr Trp Glu Glu Ser Leu Leu Ala Cys Thr Ser Lys	
120 125 130	
AAC TCC AGT CTG CTT TCT ATA GAT AAT GAA GAA GAA ATG AAA TTT CTG	608
Asn Ser Ser Leu Leu Ser Ile Asp Asn Glu Glu Glu Met Lys Phe Leu	
135 140 145	
TCC ATC ATT TCA CCA TCC TCA TGG ATT GGT GTG TTT CGT AAC AGC AGT	656
Ser Ile Ile Ser Pro Ser Ser Trp Ile Gly Val Phe Arg Asn Ser Ser	
150 155 160	
CAT CAT CCA TGG GTG ACA ATG AAT GGT TTG GCT TTC AAA CAT GAG ATA	704
His His Pro Trp Val Thr Met Asn Gly Leu Ala Phe Lys His Glu Ile	
165 170 175 180	
AAA GAC TCA GAT AAT GCT GAA CTT AAC TGT GCA GTG CTA CAA GTA AAT	752
Lys Asp Ser Asp Asn Ala Glu Leu Asn Cys Ala Val Leu Gln Val Asn	
185 190 195	
CGA CTT AAA TCA GCC CAG TGT GGA TCT TCA ATA ATA TAT CAT TGT AAG	800
Arg Leu Lys Ser Ala Gln Cys Gly Ser Ser Ile Ile Tyr His Cys Lys	
200 205 210	
CAT AAG CTT TAGAGGTAAA GCGTTTGCAT TTGCAGTGCA TCAGATAAAT	849
His Lys Leu	
215	
TGTATATTTT TTAAATAGA AATATATTAT GATTGCATAA ATCTTAAAT GAATTATGTT	909
ATTTGCTCTA ATAAGAAAAT TCTAAATCAA TTATTGAAAC AGGATACACA CAATTACTAA	969
AGTACAGACA TCCTAGCATT TGTGTCGGGC TCATTTTGCT CAACATGGTA TTTGTGGTTT	1029
TCAGCCTTTC TAAAAGTTGC ATGTTATGTG AGTCAGCTTA TAGGAAGTAC CAAGAACAGT	1089
CAAACCCATG GAGACAGAAA GTAGAATAGT GGTTGCCAAT GTCTGAGGGA GGTGAAATA	1149
GGAGATGACC TCTAACTGAT AGAACGTTAC TTTGTGTCGT GATGAAAAC TTCTAAATTT	1209

CAGTAGTGGT GATGGTTGTA ACTCTGCGAA TATACTAAAC ATCATTGATT TTTAATCATT 1269
 TTAAGTGCAT GAAATGTATG CTTTGTACAC GACACTTCAA TAAAGCTATC CAGAAAAAAA 1329
 AAAA 1333

(2) INFORMATION FOR SEQ ID NO:16:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 215 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Met	Asp	Asn	Gln	Gly	Val	Ile	Tyr	Ser	Asp	Leu	Asn	Leu	Pro	Pro	Asn	1	5	10	15
Pro	Lys	Arg	Gln	Gln	Arg	Lys	Pro	Lys	Gly	Asn	Lys	Ser	Ser	Ile	Leu	20	25	30	
Ala	Thr	Glu	Gln	Glu	Ile	Thr	Tyr	Ala	Glu	Leu	Asn	Leu	Gln	Lys	Ala	35	40	45	
Ser	Gln	Asp	Phe	Gln	Gly	Asn	Asp	Lys	Thr	Tyr	His	Cys	Lys	Asp	Leu	50	55	60	
Pro	Ser	Ala	Pro	Glu	Lys	Leu	Ile	Val	Gly	Ile	Leu	Gly	Ile	Ile	Cys	65	70	75	80
Leu	Ile	Leu	Met	Ala	Ser	Val	Val	Thr	Ile	Val	Val	Ile	Pro	Ser	Arg	85	90	95	
His	Cys	Gly	His	Cys	Pro	Glu	Glu	Trp	Ile	Thr	Tyr	Ser	Asn	Ser	Cys	100	105	110	
Tyr	Tyr	Ile	Gly	Lys	Glu	Arg	Arg	Thr	Trp	Glu	Glu	Ser	Leu	Leu	Ala	115	120	125	
Cys	Thr	Ser	Lys	Asn	Ser	Ser	Leu	Leu	Ser	Ile	Asp	Asn	Glu	Glu	Glu	130	135	140	
Met	Lys	Phe	Leu	Ser	Ile	Ile	Ser	Pro	Ser	Ser	Trp	Ile	Gly	Val	Phe	145	150	155	160

Arg Asn Ser Ser His His Pro Trp Val Thr Met Asn Gly Leu Ala Phe
 165 170 175
 Lys His Glu Ile Lys Asp Ser Asp Asn Ala Glu Leu Asn Cys Ala Val
 180 185 190
 Leu Gln Val Asn Arg Leu Lys Ser Ala Gln Cys Gly Ser Ser Ile Ile
 195 200 205
 Tyr His Cys Lys His Lys Leu
 210 215

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 135 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: C-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Gln Arg His Asn Asn Ser Ser Leu Asn Thr Arg Thr Gln Lys Ala Arg
 1 5 10 15
 His Cys Gly His Cys Pro Glu Glu Trp Ile Thr Tyr Ser Asn Ser Cys
 20 25 30
 Tyr Tyr Ile Gly Lys Glu Arg Arg Thr Trp Glu Glu Ser Leu Leu Ala
 35 40 45
 Cys Thr Ser Lys Asn Ser Ser Leu Leu Ser Ile Asp Asn Glu Glu Glu
 50 55 60
 Met Lys Phe Leu Ser Ile Ile Ser Pro Ser Ser Trp Ile Gly Val Phe
 65 70 75 80

Arg Asn Ser Ser His His Pro Trp Val Thr Met Asn Gly Leu Ala Phe
 85 90 95
 Lys His Glu Ile Lys Asp Ser Asp Asn Ala Glu Leu Asn Cys Ala Val
 100 105 110
 Leu Gln Val Asn Arg Leu Lys Ser Ala Gln Cys Gly Ser Ser Ile Ile
 115 120 125
 Tyr His Cys Lys His Lys Leu
 130 135

(2) INFORMATION FOR SEQ ID NO:18:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 102 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: C-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Tyr Ile Gly Lys Glu Arg Arg Thr Trp Glu Glu Ser Leu Leu Ala Cys
 1 5 10 15
 Thr Ser Lys Asn Ser Ser Leu Leu Ser Ile Asp Asn Glu Glu Glu Met
 20 25 30
 Lys Phe Leu Ser Ile Ile Ser Pro Ser Ser Trp Ile Gly Val Phe Arg
 35 40 45
 Asn Ser Ser His His Pro Trp Val Thr Met Asn Gly Leu Ala Phe Lys
 50 55 60
 His Glu Ile Lys Asp Ser Asp Asn Ala Glu Leu Asn Cys Ala Val Leu
 65 70 75 80

Gln Val Asn Arg Leu Lys Ser Ala Gln Cys Gly Ser Ser Ile Ile Tyr
 85 90 95

His Cys Lys His Lys Leu
 100

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 135 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: C-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Leu Glu Gln Asn Asn Ser Ser Pro Asn Thr Arg Thr Gln Lys Ala Arg
 1 5 10 15

His Cys Gly His Cys Pro Glu Glu Trp Ile Thr Tyr Ser Asn Ser Cys
 20 25 30

Tyr Tyr Ile Gly Lys Glu Arg Arg Thr Trp Glu Glu Ser Leu Leu Ala
 35 40 45

Cys Thr Ser Lys Asn Ser Ser Leu Leu Ser Ile Asp Asn Glu Glu Glu
 50 55 60

Ile Lys Phe Leu Ala Ser Ile Leu Pro Ser Ser Trp Ile Gly Val Phe
 65 70 75 80

Arg Asn Ser Ser His His Pro Trp Val Thr Ile Asn Gly Leu Ala Phe
 85 90 95

Lys His Lys Ile Lys Asp Ser Asp Asn Ala Glu Leu Asn Cys Ala Val
 100 105 110

Leu Gln Val Asn Arg Leu Lys Ser Ala Gln Cys Gly Ser Ser Met Ile
 115 120 125
 Tyr His Cys Lys His Lys Leu
 130 135

(2) INFORMATION FOR SEQ ID NO:20:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 134 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: C-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Val Gln Ile Pro Leu Val Ala Ile Trp Thr Glu Ser Tyr Cys Gly Pro
 1 5 10 15
 Cys Pro Lys Asn Asn Cys Tyr Gln Phe Phe Asp Glu Ser Lys Asn Trp
 20 25 30
 Tyr Glu Ser Gln Ser Cys Met Ser Gln Asn Ala Ser Leu Leu Lys Val
 35 40 45
 Tyr Ser Lys Glu Gln Asp Leu Leu Lys Leu Val Lys Ser Tyr His Trp
 50 55 60
 Met Gly Leu Val His Asp Ala Trp Ile Cys Tyr Lys Asn Ile Pro Thr
 65 70 75 80
 Asn Gly Ser Trp Gln Trp Glu Asp Gly Ser Ile Leu Ser Pro Asn Leu
 85 90 95
 Leu Thr Ile Ile Glu Met Gln Lys Gly Asp Cys Ala Leu Tyr Ala Ser
 100 105 110

Ser Phe Lys Gly Tyr Ile Glu Asn Cys Ser Thr Pro Asn Thr Tyr Ile
 115 120 125

Cys Met Gln Arg Thr Val
 130

(2) INFORMATION FOR SEQ ID NO:21:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 233 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: C-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

Met Asp Asn Gln Gly Val Ile Tyr Ser Asp Leu Asn Leu Pro Pro Asn
 1 5 10 15

Pro Lys Arg Gln Gln Arg Lys Pro Lys Gly Asn Lys Ser Ser Ile Leu
 20 25 30

Ala Thr Glu Gln Glu Ile Thr Tyr Ala Glu Leu Asn Leu Gln Lys Ala
 35 40 45

Ser Gln Asp Phe Gln Gly Asn Asp Lys Thr Tyr His Cys Lys Asp Leu
 50 55 60

Pro Ser Ala Pro Glu Lys Leu Ile Val Gln Ile Leu Gln Ile Ile Cys
 65 70 75 80

Ile Ile Leu Met Ala Ser Val Val Thr Ile Val Val Ile Pro Ser Thr
 85 90 95

Leu Ile Gln Arg His Asn Asn Ser Ser Leu Asn Thr Arg Thr Gln Lys
 100 105 110

Ala Arg His Cys Gly His Cys Pro Glu Glu Trp Ile Thr Tyr Ser Asn
115 120 125
Ser Cys Tyr Tyr Ile Gly Lys Glu Arg Arg Thr Trp Glu Glu Ser Leu
130 135 140
Leu Ala Cys Thr Ser Lys Asn Ser Ser Leu Leu Ser Ile Asp Asn Glu
145 150 155 160
Glu Glu Met Lys Phe Leu Ser Ile Ile Ser Pro Ser Ser Trp Ile Gly
165 170 175
Val Phe Arg Asn Ser Ser His His Pro Trp Val Thr Met Asn Gly Leu
180 185 190
Ala Phe Lys His Glu Ile Lys Asp Ser Asp Asn Ala Glu Leu Asn Cys
195 200 205
Ala Val Leu Gln Val Asn Arg Leu Lys Ser Ala Gln Cys Gly Ser Ser
210 215 220
Ile Ile Tyr His Cys Lys His Lys Leu
225 230

(2) INFORMATION FOR SEQ ID NO:22:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 215 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: C-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

Met	Asp	Asn	Gln	Gly	Val	Ile	Tyr	Ser	Asp	Leu	Asn	Leu	Pro	Pro	Asn	1	5	10	15
Pro	Lys	Arg	Gln	Gln	Arg	Lys	Pro	Lys	Gly	Asn	Lys	Ser	Ser	Ile	Leu	20	25	30	
Ala	Thr	Glu	Gln	Glu	Ile	Thr	Tyr	Ala	Glu	Leu	Asn	Leu	Gln	Lys	Ala	35	40	45	
Ser	Gln	Asp	Phe	Gln	Gly	Asn	Asp	Lys	Thr	Tyr	His	Cys	Lys	Asp	Leu	50	55	60	
Pro	Ser	Ala	Pro	Glu	Lys	Leu	Ile	Val	Gly	Ile	Leu	Gly	Ile	Ile	Cys	65	70	75	80
Leu	Ile	Leu	Met	Ala	Ser	Val	Val	Thr	Ile	Val	Val	Ile	Pro	Ser	Arg	85	90	95	
His	Cys	Gly	His	Cys	Pro	Glu	Glu	Trp	Ile	Thr	Tyr	Ser	Asn	Ser	Cys	100	105	110	
Tyr	Tyr	Ile	Gly	Lys	Glu	Arg	Arg	Thr	Trp	Glu	Glu	Ser	Leu	Leu	Ala	115	120	125	
Cys	Thr	Ser	Lys	Asn	Ser	Ser	Leu	Leu	Ser	Ile	Asp	Asn	Glu	Glu	Glu	130	135	140	
Met	Lys	Phe	Leu	Ser	Ile	Ile	Ser	Pro	Ser	Ser	Trp	Ile	Gly	Val	Phe	145	150	155	160

Arg	Asn	Ser	Ser	His	His	Pro	Trp	Val	Thr	Met	Asn	Gly	Leu	Ala	Phe
				165					170					175	
Lys	His	Glu	Ile	Lys	Asp	Ser	Asp	Asn	Ala	Glu	Leu	Asn	Cys	Ala	Val
			180					185					190		
Leu	Gln	Val	Asn	Arg	Leu	Lys	Ser	Ala	Gln	Cys	Gly	Ser	Ser	Ile	Ile
		195					200					205			
Tyr	His	Cys	Lys	His	Lys	Leu									
	210					215									

(2) INFORMATION FOR SEQ ID NO:23:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 231 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (v) FRAGMENT TYPE: C-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

Met	Ser	Lys	Gln	Arg	Gly	Thr	Phe	Ser	Glu	Val	Ser	Leu	Ala	Gln	Asp
1			5						10					15	
Pro	Leu	Arg	Gln	Gln	Arg	Lys	Pro	Lys	Gly	Asn	Lys	Ser	Ser	Ile	Ser
			20					25					30		
Gly	Thr	Glu	Glu	Glu	Ile	Phe	Gln	Val	Glu	Leu	Asn	Leu	Gln	Asn	Pro
		35				40						45			
Ser	Leu	Asn	His	Gln	Gly	Ile	Asp	Lys	Ile	Tyr	Asp	Cys	Gln	Gly	Leu
	50					55					60				
Leu	Pro	Pro	Pro	Glu	Lys	Leu	Thr	Ala	Glu	Val	Leu	Gly	Ile	Ile	Cys
65					70					75					80

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Ile Val Leu Met Ala Thr Val Leu Lys Thr Ile Val Leu Ile Pro Phe
      85                      90                      95
Leu Glu Gln Asn Asn Ser Ser Pro Asn Thr Arg Thr Gln Lys Ala Arg
      100                      105                      110
His Cys Gly His Cys Pro Glu Glu Thr Ile Thr Tyr Ser Asn Ser Cys
      115                      120                      125
Tyr Tyr Ile Gly Lys Glu Arg Arg Thr Trp Glu Glu Ser Leu Leu Ala
      130                      135                      140
Cys Thr Ser Lys Asn Ser Ser Leu Leu Ser Ile Asp Asn Glu Glu Glu
      145                      150                      155                      160
Ile Lys Phe Leu Ala Ser Ile Leu Pro Ser Ser Trp Ile Gly Val Phe
      165                      170                      175
Arg Asn Ser Ser His His Pro Trp Val Thr Ile Asn Gly Leu Ala Phe
      180                      185                      190
Lys His Lys Ile Lys Asp Ser Asp Asn Ala Glu Leu Asn Cys Ala Val
      195                      200                      205
Leu Gln Val Asn Arg Leu Lys Ser Ala Gln Cys Gly Ser Ser Met Ile
      210                      215                      220
Tyr His Cys Lys His Lys Leu
      225                      230

```

(2) INFORMATION FOR SEQ ID NO:24:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 216 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: protein

- (iii) HYPOTHETICAL: NO

- (iv) ANTI-SENSE: NO

- (v) FRAGMENT TYPE: C-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

Met Gly Trp Ile Arg Gly Arg Arg Ser Arg His Ser Trp Glu Met Ser
 1 5 10 15
 Glu Phe His Asn Tyr Asn Leu Asp Leu Lys Lys Ser Asp Phe Ser Thr
 20 25 30
 Arg Trp Gln Lys Gln Arg Cys Pro Val Val Lys Ser Lys Cys Arg Glu
 35 40 45
 Asn Ala Ser Pro Phe Phe Phe Cys Cys Phe Ile Ala Val Ala Met Gly
 50 55 60
 Ile Arg Phe Ile Ile Met Ser Ala Val Phe Leu Asn Ser Leu Phe Asn
 65 70 75 80
 Gln Glu Val Gln Ile Pro Leu Val Ala Ile Trp Thr Glu Ser Tyr Cys
 85 90 95
 Gly Pro Cys Pro Lys Asn Asn Cys Tyr Gln Phe Phe Asp Glu Ser Lys
 100 105 110
 Asn Thr Tyr Glu Ser Gln Ser Cys Met Ser Gln Asn Ala Ser Leu Leu
 115 120 125
 Lys Val Tyr Ser Leu Glu Gln Asp Leu Leu Lys Leu Val Lys Ser Tyr
 130 135 140
 His Trp Met Gly Leu Val His Asp Ala Trp Ile Cys Tyr Lys Asn Ile
 145 150 155 160
 Pro Thr Asn Gly Ser Trp Gln Trp Glu Asp Gly Ser Ile Leu Ser Pro
 165 170 175
 Asn Leu Leu Thr Ile Ile Glu Met Asn Lys Gly Asp Cys Ala Leu Tyr
 180 185 190
 Ala Ser Ser Phe Lys Gly Tyr Ile Glu Asn Cys Ser Thr Pro Asn Thr
 195 200 205
 Tyr Ile Cys Met Gln Arg Thr Val
 210 215

CLAIMS:

1. An isolated DNA or cDNA sequence encoding the extracellular part of a transmembrane protein herein designated (a) NKG2-A_f, (b) NKG2-B_f, (c) NKG2-C_f and (d) NKG2-D_f (f = fragment) translated in natural killer cells or T cells, wherein the DNA or cDNA sequence is selected from the group comprising:
 - (a) that part of the DNA sequence shown in Seq. Id. No.: 1, comprising the nucleotides numbered 458 to 863 inclusive (= the entire sequence shown in Seq. Id. No.: 3); or
 - (b) that part of the DNA sequence shown in Seq. Id. No. 1, comprising the nucleotides numbered 503 to 863 inclusive (= the entire sequence shown in Seq. Id. No. 4); or
 - (c) that part of the DNA sequence shown in Seq. Id. No.: 5 comprising the nucleotides numbered 296 to 700 inclusive (= the entire sequence shown in Seq. Id. No.: 7); or
 - (d) that part of the DNA sequence shown in Seq. Id. No.: 8, comprising the nucleotides numbered 585 to 986 inclusive (= the entire sequence shown in Seq. Id. No.: 10); or
 - (e) DNA or cDNA sequences that code for the same amino acid sequence as that coded for by any of (a)-(d) above; and/or which hybridise under stringent conditions to any of (a)-(d) above; and/or have a homology of 80 to 100% with any of the DNA sequences (a)-(d) above.
2. An isolated DNA or cDNA sequence encoding a complete transmembrane protein herein designated aa) NKG2-A, bb) NKG2-B, cc) NKG2-C and dd) NKG2-D which are translated in natural killer cells or T cells, wherein the DNA or cDNA sequence is selected from the group

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comprising:

- aa) that part of the DNA sequence shown in Seq. Id. No.: 1, comprising the nucleotides numbered 165 to 863 inclusive (= the entire sequence shown in Seq. Id. No.: 11), or
- bb) that part of the DNA sequence shown in Seq. Id. No. 1, comprising the nucleotides numbered 165 to 863 inclusive; the nucleotides numbered 449 to 502 being deleted (= the entire sequence shown in Seq. Id. No.: 12); or
- cc) that part of the DNA sequence shown in Seq. Id. No.: 5 comprising the nucleotides numbered 8 to 700 inclusive (= the entire sequence shown in Seq. Id. No.: 13); or
- dd) that part of the DNA sequence shown in Seq. Id. No.: 8, comprising the nucleotides numbered 339 to 986 inclusive (= the entire sequence shown in Seq. Id. No.: 14); or
- ee) isolated DNA or cDNA sequences that code for the same amino acid sequence as that coded for by any of aa)-dd) above; and/or which hybridise under stringent conditions to any of aa)-dd) above; and/or have a homology of 80 to 100% with any of the DNA sequences aa)-dd) above.

3. The isolated extracellular part of a transmembrane protein designated a') NKG2-A_f, b') NKG2-B_f, c') NKG2-C_f and d') NKG2-D_f having an amino acid sequence selected from the group comprising:

- a') that part of the amino acid sequence in Seq. Id. Nos.: 1 and 2 comprising the amino acids numbered 99 to 223 (= the entire amino acid sequence of Seq. Id. No.: 17); or
- b') that part of the amino acid sequence in Seq. Id. No.: 1 comprising the amino acids numbered 114 to 233 (= the entire amino acid sequence of Seq. Id. No.: 18); or

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- c') that part of the amino acid sequence in Seq. Id. No.: 5 comprising the amino acids numbered 97 to 231 (= the entire amino acid sequence of Seq. Id. No.: 19); or
- d') that part of the amino acid sequence in Seq. Id. No.: 9 comprising the amino acids numbered 83 to 216 (= the entire amino acid sequence of Seq. Id. No.: 20); or
- e') sequence variants, as herein defined, of any of the above.

4. An isolated complete transmembrane protein designated aa') NKG2-A, bb') NKG2-B, cc') NKG2-C and dd') NKG2-D, having an amino acid sequence selected from the group comprising:

- aa') the amino acid sequence in Seq. Id. No.: 2 (or Seq. Id. No.: 21); or
- bb') the amino acid sequence in Seq. Id. No.: 2 with the deletion of the amino acids numbered 96 to 113 (or Seq. Id. No.: 22); or
- cc') the amino acid sequence in Seq. Id. No.: 6 (or Seq. Id. No.: 23); or
- dd') the amino acid sequence in Seq. Id. No.: 8 (or Seq. Id. No.: 24); or
- ee') sequence variants, as herein defined, of any of the above.

5. A polyclonal or monoclonal antibody recognising at least one epitope of a protein according to Claims 3 or 4.

6. A bifunctional antibody recognising a) at least one epitope of a protein according to Claims 3 or 4 and b) a cancer- or virus-specific

antigen.

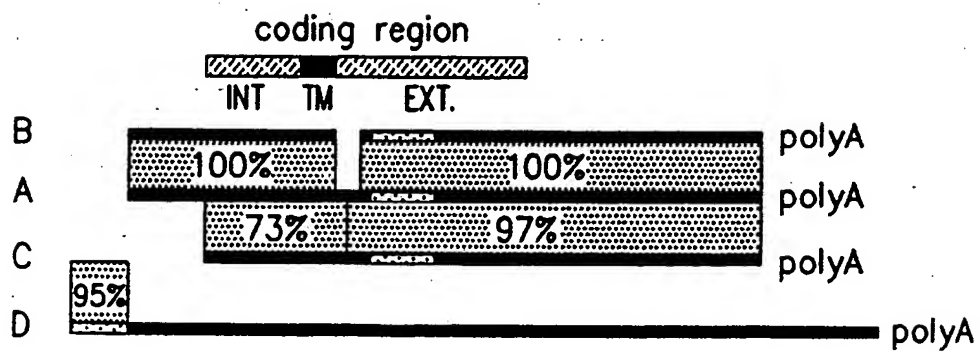
7. Treatment of cancer or virus infection in a human in need of such treatment by administering an effective dose of a bifunctional antibody according to Claim 6.

8. A chimeric protein molecule comprising a protein according to Claim 3 linked to a cytotoxic protein.

9. Treatment of cancer or virus infection in a human in need of such treatment by administering an effective dose of a chimeric protein according to Claim 8.

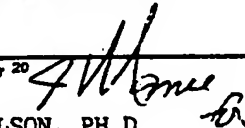
10. Method of treating cancer or virus infection in a human in need of such treatment comprising the steps of a) removing NK cells from the patient to be treated, b) transforming the cells in vitro with DNA sequences according to Claim 2 and c) reinjecting the transformed cells into the patient.

FIG. 1



INTERNATIONAL SEARCH REPORT

International Application No. PCT/US92/02469

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) ³		
According to International Patent Classification (IPC) or to both National Classification and IPC		
IPC (5): A61K 37/10 US CL : 514/8		
II. FIELDS SEARCHED		
Minimum Documentation Searched ⁴		
Classification System	Classification Symbols	
U.S.	514/8	
Documentation Searched other than Minimum Documentation to the extent that such Documents are included in the Fields Searched ⁵		
APS, Dialog, Intelligenetics		
III. DOCUMENTS CONSIDERED TO BE RELEVANT ¹⁴		
Category ⁶	Citation of Document, ¹⁸ with indication, where appropriate, of the relevant passages ¹⁷	Relevant to Claim No. ¹⁸
X/Y,P	J. Exp. Med., Vol. 173, issued April 1991, J.P. Houchins et al., "DNA Sequence Analysis of NKG2, a family of related cDNA clones encoding type II integral membrane proteins on human natural killer cells", pages 1017-1020, see entire document.	1-5,6,8/7-10
Y,P	FASEB J., Vol. 5, No. 5, issued 1991, J.P. Houchins et al., "NKG2: A multigene family on human chromosome 12 that encodes NK-specific, lectin-like transmembrane proteins", page A1099, see entire abstract.	1-10
Y	J. Mol. Cell. Immunol., Vol. 4, issued 1990, J.P. Houchins et al., "Isolation and characterization of NK cell or NK/T cell-specific cDNA clones", pages 295-306, see entire document.	1-10
Y	J. Biol. Chem., Vol. 263, No. 20, issued 15 July 1988, K. Drickamer, "Two distinct classes of carbohydrate-recognition domains in animal lectins", pages 9557-9560, see entire document.	1-10
<p>⁶ Special categories of cited documents:¹⁵</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (see specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</p> <p>"A" document member of the same patent family</p>		
IV. CERTIFICATION		
Date of the Actual Completion of the International Search ²		Date of Mailing of this International Search Report ²
17 JUNE 1992		20 JUN 1992
International Searching Authority ¹		Signature of Authorized Officer ²⁰
ISA/US		KAREN COCHRANE CARLSON, PH.D. 

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

Y	J. Immunol., Vol. 143, No. 4, issued 15 August 1989, W.M. Yokoyama et al., "A murine T lymphocyte antigen belongs to a supergene family of type II integral membrane proteins", pages 1379-1386, see entire document.	1-10
Y	Science, Vol. 249, issued 14 September 1990, R. Giorda et al., "NKR-P1, a signal transduction molecule on natural killer cells", pages 1298-1300, see entire document.	1-10

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE¹

1. ☐ Claim numbers , because they relate to subject matter (1) not required to be searched by this Authority, namely:
2. ☐ Claim numbers , because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out (1), specifically:
3. ☐ Claim numbers , because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING²

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.
2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:
3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:
4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Search Authority did not invite payment of any additional fee.

Remark on protest

- ☐ The additional search fees were accompanied by applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.